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4. TITLE AND SUBTITLE
Archive of Samples for Long-term Preservation of RNA and other nucleic acids

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5d. PROJECT NUMBER

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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The project's objective was the development of a scalable all ambient temperature biological sample workflow preserving nucleic acids for molecular analysis. This ambient workflow can be developed for large scale blood collection, transport, and long-term archiving without the use of costly and unreliable cold chain management, using commercially available biopreservation products. For the development of the automated workflow we have chosen Biomatrica's commercially validated products RNAgard Blood tubes, RNAstable and DNAstable Plus, an off the shelf liquid handler (Beckman), a commercially available dryer (Genevac Inc) and small 384 well storage tubes with a commercial tube sealing system for compact storage (Thermo). In this research period we have achieved the final Phase II milestone of processing 500 samples n a week.

15. SUBJECT TERMS
Nucleic Acid Preservation, Cost Reduction, Ambient Temperature Stabilization, Molecular Diagnostic, Biobank, SOP (Standard Operating Procedure), Workflow, Disease Prevention

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TITLE: Archive of Samples for Long-term Preservation of RNA and other nucleic acids

PRINCIPAL INVESTIGATOR: Vasco Liberal

CONTRACTING ORGANIZATION: Biomatrica

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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1. **INTRODUCTION:** The project's objective is the development of a scalable all ambient temperature biological sample workflow preserving the nucleic acids for molecular analysis. This ambient workflow can be developed for large scale blood collection, transport and long-term nucleic acid archiving without the use of costly and unreliable cold-chain management, using commercially available biopreservation products. In Phase I we successfully demonstrated the power of chemical ambient preservation of nucleic acids throughout the workflow from blood sample collection and transport to the reliable storage of nucleic acids. The Phase II development will result in the integration of automation equipment, the establishment of Standard Operating Procedures (SOPs) and an optimized workflow that reduces operational costs. The automated methods can be directly implemented for blood sample processing and archiving of nucleic acids in a routine setting.

2. **KEYWORDS:** Nucleic Acid Preservation, Cost Reduction, Ambient Temperature Stabilization, Molecular Diagnostic, Biobank, SOP (Standard Operating Procedure), Workflow, Disease Prevention

3. **ACCOMPLISHMENTS:**

What were the major goals of the project? This Phase II project has 5 main technical objectives:

1. Systems Review – occurred throughout the entire project, completed.
2. Automated workflow development – began and completed in the first year of Phase II.
3. Workflow implementation and through-put scale up – this objective began in quarter 3 of year 1, and proceeded to completion in year 2 of the project.
4. Cost analysis for automated workflow – immediately initiated and completed upon finalization of technical objective 3.
5. Documentation - initiated and completed upon finalization of technical objective 3.

What was accomplished under these goals? As described in the previous annual report, the first year of this project represented a significant amount of progress towards satisfying the goals of this project, most specifically in completion of technical objectives 2 and the initiation of technical objective 3. At the point where this previous annual report concluded, we were continuing to optimize automated purification chemistries to meet nucleic acid yield and purity, as well as sample through-put specifications for completion of technical objective 3 (Table 1).

Army Requirement	RNA	DNA
Nucleic acid yield	**5 ug (4 wells x 1.25 ug)**	**12 ug (4 wells x 3 ug)**
Purity (A260/A280)	**1.8-2.2**	**1.8-2.2**
Sample storage	**4 replicates in 384 well plate**	**4 replicates in 384 well plate**

Table 1. Army requirements for yield and purity of nucleic acids purified from blood collected in RNAgard blood tubes.

This process continued into the timeline covered by this report. For RNA processing, we selected the Agencourt RNAdvance Blood method on the Biomek FX platform. For DNA sample processing the automated hardware was changed from Biomek FX to the KingFisher Flex, a rod-based magnetic purification module which is produced by ThermoFisher. We found that this instrument was best compatible with purifying DNA from RGB supernatant via Macherey-Nagel NucleoMag Blood kit. Following a short manual process in which samples from RNAgard Blood (RGB) tubes are centrifuged to separate the sample into two phases that their respective RNA and DNA components will be isolated from, each fraction then proceeds to a nearly fully automated nucleic acid purification followed by subsequent manual quantification, drying, and storage processes (Figure 1).

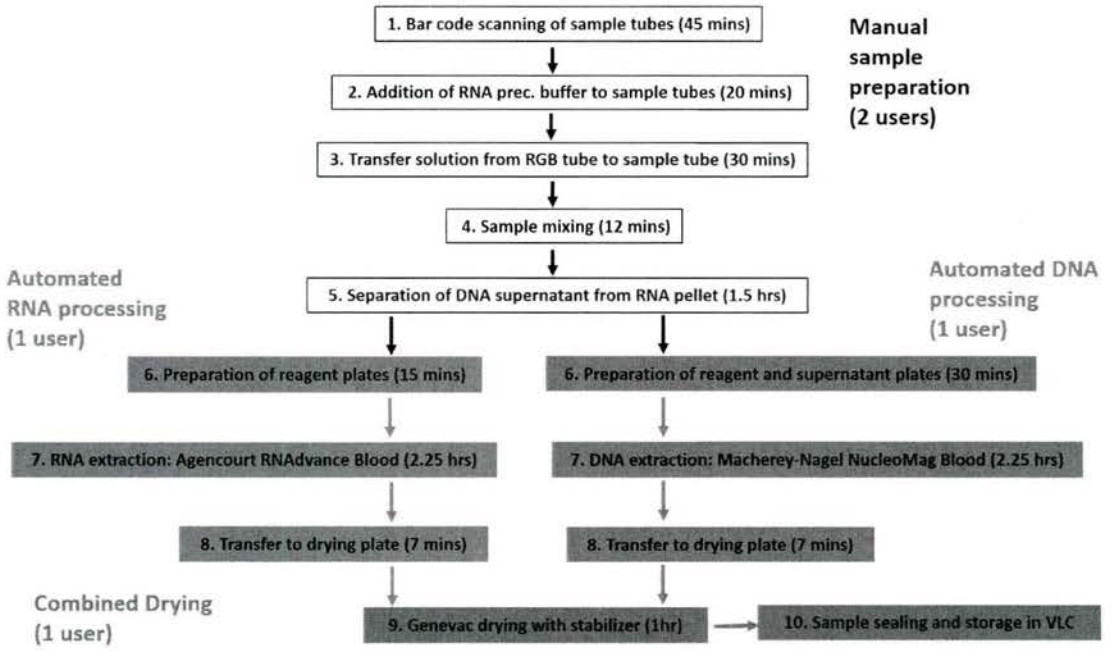


Figure 1. Workflow of automated nucleic acid purification and storage from of blood samples collected in RNAgard Blood tubes. After a manual process in which the samples are separated into supernatant and pellet by 2 users (black), DNA is purified from the supernatant by a single user (blue), while the other user purifies RNA from the pellet (orange). Once these nucleic acid purification workflows are completed, the samples from each process are dried and archived (red).

With specific regard to optimizing the DNA purification process, although purification of small volumes (320 uL or less) of RNAgard Blood supernatant proved to be fruitful in regards to DNA yield, we found that the yield achievable from these small scale purifications did not scale up in a linear fashion when sample volume was increased. In particular, the greatest hindrance to this process was noticed when the increase in sample volume necessitated use of a 24-well sample plate rather than a 96-well sample plate. An additional inconvenience was that large volume purifications carried out in 24-well plates also sometimes carried along a red coloration that proved difficult to remove. However, we found that by splitting larger sample volumes into multiple 96-well plates rather than a single 24-well plate (for example, 1920 uL of RNAgard Blood supernatant is split and purified as 6 individual wells of 320 uL), DNA yield and purity specifications were achieved much more consistently and that yield variability observed from

sample to sample that was collected from the same donor also decreased. Coloration effects observed in the larger samples are also absent when the purification process is carried out in 96-well samples plates. To alleviate any concern that DNA yields might be lower when samples of 1920 uL are split 6 ways among a larger volume of magnetic beads (75 uL), we compared the yields of this process to the summed yields of 6 individual 320 uL samples that are each purified with 12.5 uL of magnetic beads (Figure 2A). By this process we found that the yields of a large 1920 uL split sample was similar to yields obtained from the sum of 6 individual 320 uL samples (Figure 2B). Importantly, we found that the variation in yield between 8 individual 320 uL replicate samples was very low, supporting the high robustness of this purification process (Figure 2C).

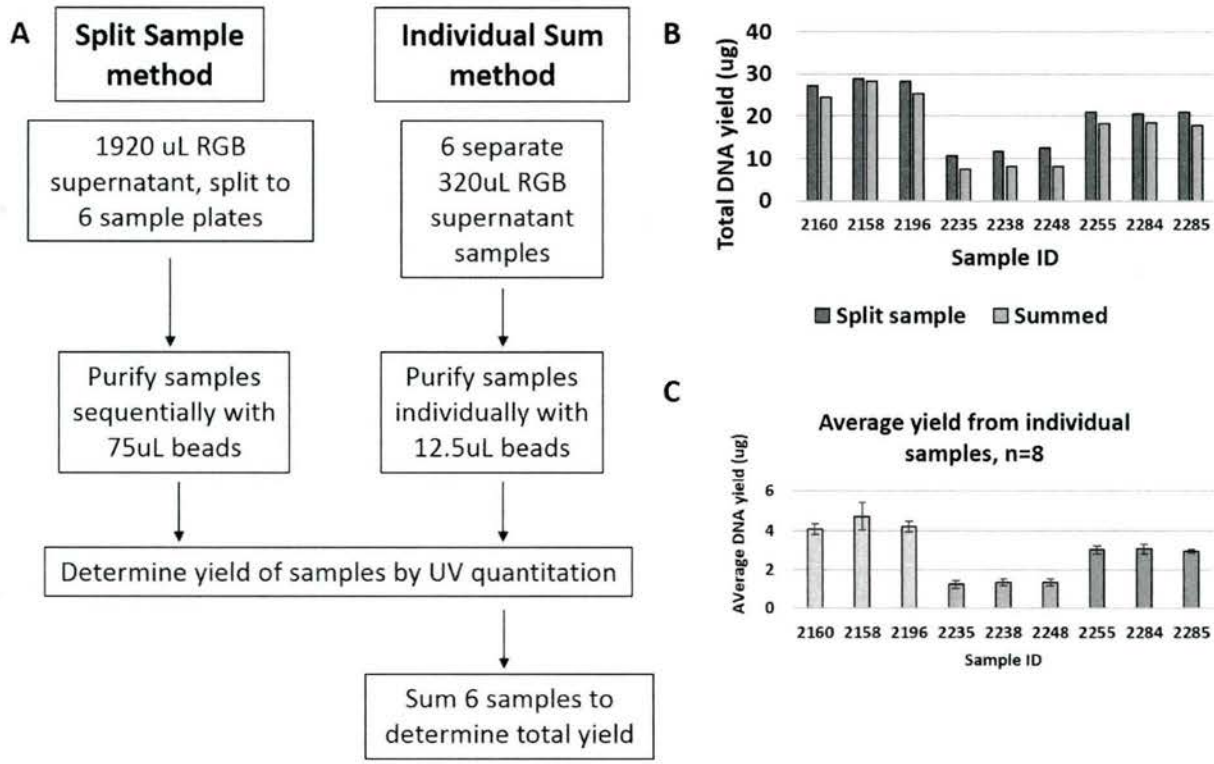


Figure 2. A) Workflow synopsis of each method that was compared. B) DNA yield comparison of a single 1920 uL split sample versus the sum of 6 individual 320 uL samples. C) Average DNA yield from 8 replicate 320 uL samples taken from 9 samples tubes.

Upon selection of the most appropriate chemistries and sample processing methods to consistently meet the proposal requirements for RNA/DNA yield and purity of this project, workflow implementation and scaling up began (objective 3). This consisted of completing a series of scaled sample purifications that demonstrated increased throughput as experimental milestones of 20 samples per week, 100 samples per week, and finally 500 samples per week are met. Results and statistics for a sample set in which 480 donor samples were isolated over the course of 5 days are shown here. For RNA isolations, our process meets the yield and purity specifications required by the Army in 94.4% and 100% of the time, respectively (Table 2). In most cases where the RNA yield specification was not met, these samples corresponded to particular donors who represented a subpopulation having generally low levels of RNA in their

blood (Figure 3,example donor 55). RNA quality, as determined on an Agilent Bioanalyzer, was high immediately following purification (i.e. RIN scores >7), and this quality was maintained after the samples were dried in the presence of RNAstable and then rehydrated after storage for 1 month at 45°C (Figure 4).

	Total	Day 1	Day 2	Day 3	Day 4	Day 5
Average yield/tube (ug)	16.7	25.9	14.5	13.4	18.0	11.6
Standard deviation	**9.2**	**12.5**	**5.7**	**5.4**	**6.5**	**6**
Total samples	480	96	96	96	96	96
Total fails	27	1	6	8	1	11
Yield Success rate	94.4%	98.9%	93.7%	91.6%	98.9%	88.5%
Purity success rate	100%	100%	100%	100%	100%	100%

Table 2. Data summary of yield and purity results achieved from 480 RNA samples purified over 5 days via Agencourt RNAdvance Blood.

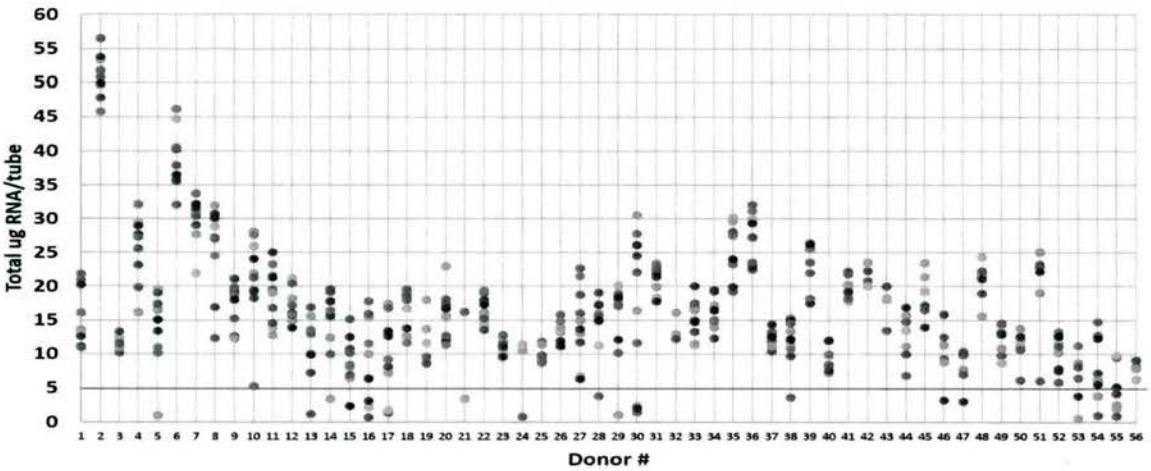
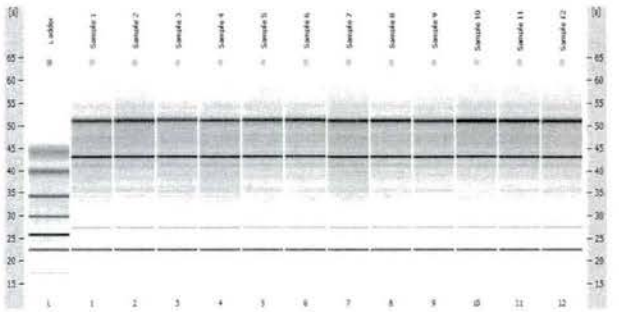
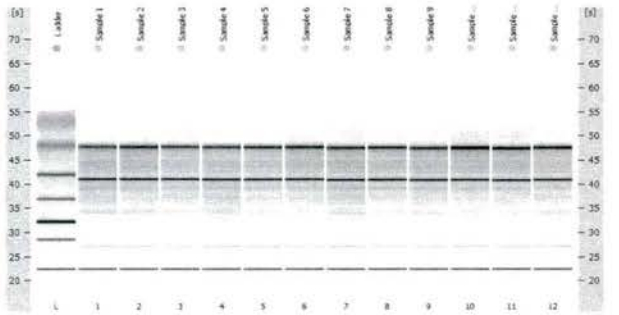


Figure 3. Profile of yield results achieved from 480 RNA samples from 54 donors purified over 5 days via Agencourt RNAdvance Blood. Red line denotes the minimum RNA yield requirement.

Day 0



1 month, 45C



Donor	Pre-dry RIN	1 month 45°C RIN
1	7.2	7.3
2	7.2	8
3	7.3	7.8
4	7.2	7.4
5	7.9	8.1
6	7.9	8.1
7	7.4	7.4
8	7.8	8
9	7.6	7.8
10	8.1	8.4
11	7.9	8.3
12	7.8	8.1

Figure 4. RNA quality is maintained following dry down with RNAstable and storage at 45°C for 1 month.

For DNA isolations, our process meets the yield and purity specifications required by the Army 89.6% and 100% of the time, respectively (Table 3). Similarly to the RNA purification process, we found that a significant amount of samples that failed to meet the DNA yield specification were clustered to a subpopulation of donors whose yields clustered near the threshold, again evidenced by testing multiple samples from the same donor (Figure 5). However, unlike the RNA purification process, in which the starting material from an entire tube is consumed, the current DNA process is utilizing 1920 uL of RNAgard Blood supernatant. Thus, for the cases of low DNA yield donors, additional material may be purified subsequently from the remaining sample (about 10 mL is left), so that the DNA yield specifications can be met. Following purification by this method, we dried DNA samples with DNAstable Plus or without protection and stored them for 3 days at 60°C. Dried samples which were stored at 4°C were included as a positive control. DNA quality was maintained in samples treated with DNAstable Plus, while non-protected (NP) samples were degraded (Figure 6).

	Total	Day 1	Day 2	Day 3	Day 4	Day 5
Average yield from 1.92 mL RGB supernatant (ug)	18.77	20.09	19.94	17.37	19.29	17.17
Standard deviation	**6.22**	**5.78**	**6.76**	**6.3**	**5.39**	**6.24**
Total samples	480	96	96	96	96	96
Total fails	50	4	8	20	6	12
Yield Success rate	89.6%	95.8%	91.7%	79.2%	93.7%	87.5%
Purity success rate	100%	100%	100%	100%	100%	100%

Table 3. Data summary of yield and purity results achieved from 480 DNA samples purified over 5 days via Macherey-Nagel NucleoMag Blood.

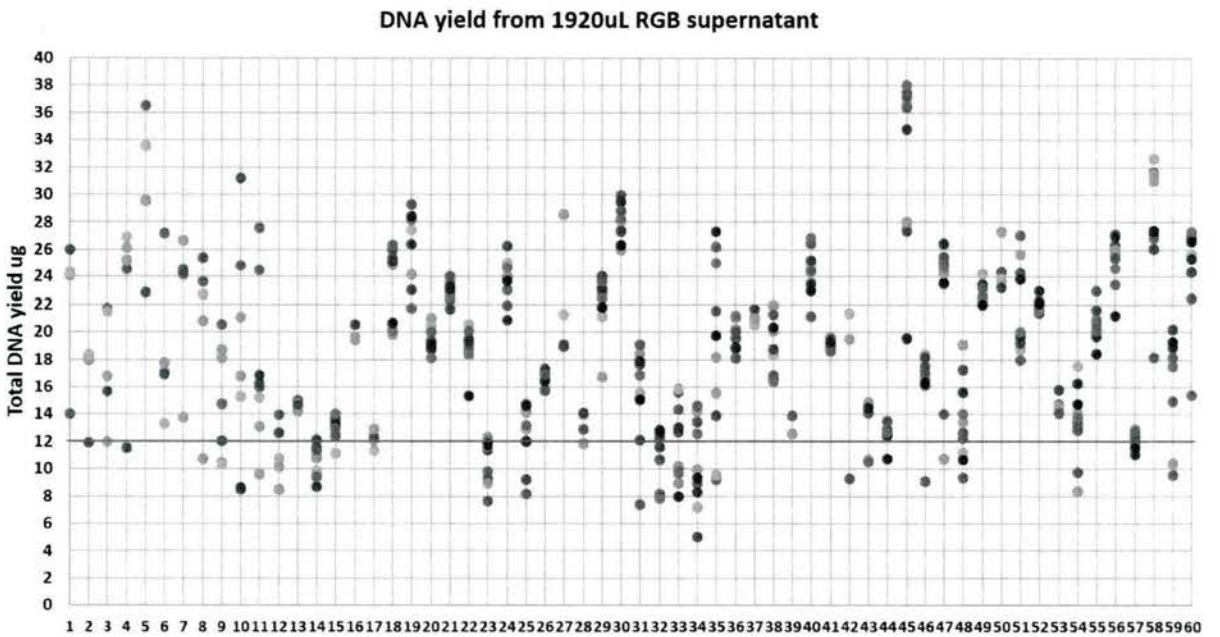
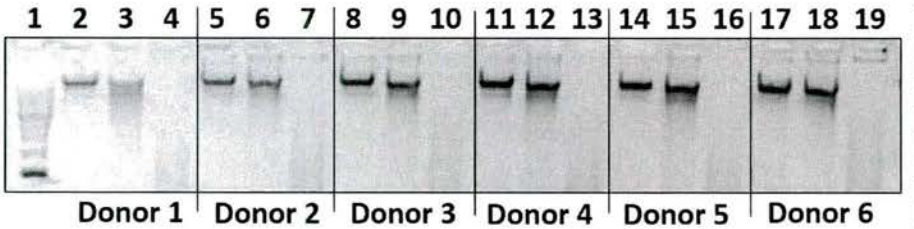


Figure 5. Profile of yield results achieved from 480 RNA samples from 60 donors extracted over 5 days via Macherey-Nagel NucleoMag Blood. Red line denotes the minimum RNA yield requirement.

Upon fulfillment of technical objective 3, cost analysis (technical objective 4) and documentation of entire study found in this report (technical objective 5) was undertaken and the results have been included as appendices in this report. As of May 15, 2016, all technical objectives have now be completed for this project (Figure 7).



Lane	Condition	Donor
1	1kb ladder	
2	DS Plus 4C	1
3	DS Plus 60C	1
4	NP 60C	1
5	DS Plus 4C	2
6	DS Plus 60C	2
7	NP 60C	2
8	DS Plus 4C	2
9	DS Plus 60C	3
10	NP 60C	3
11	DS Plus 4C	3
12	DS Plus 60C	4
13	NP 60C	4
14	DS Plus 4C	4
15	DS Plus 60C	5
16	NP 60C	5
17	DS Plus 4C	5
18	DS Plus 60C	6
19	NP 60C	6

Figure 6. Quality of DNA dried with DNAstable Plus (DS Plus) is preserved after incubation at 60°C for 3 days while degradation is observed for un-protected samples (NP). Samples were also stored at 4°C as a positive control.

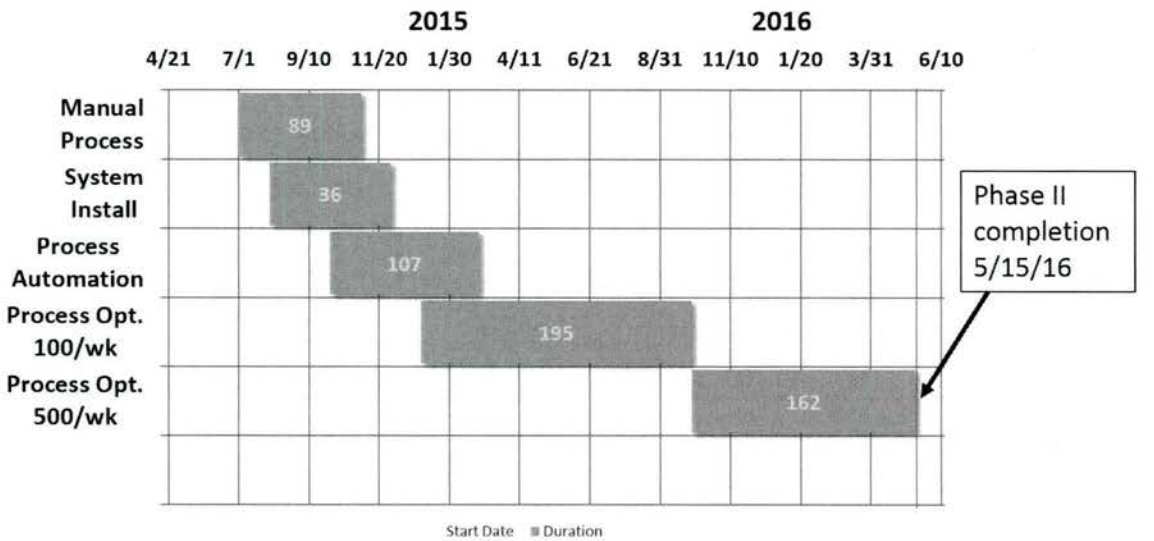


Figure 7. Timeline of Phase II milestones. Red line indicates progress to the timelines.

What opportunities for training and professional development has the project provided? This project has provided training for research associates in molecular biology techniques, automated sample processing, and workflow optimization.

How were the results disseminated to communities of interest? A poster was presented at the Society for Laboratory Automation and Screening conference in 2016.

What do you plan to do during the next reporting period to accomplish the goals? Not applicable as all the goals have been accomplished.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project? Nothing to Report

What was the impact on other disciplines? Nothing to Report

What was the impact on technology transfer? Nothing to Report

What was the impact on society beyond science and technology? Nothing to Report

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them Nothing to Report

Changes that had a significant impact on expenditures Nothing to Report

Significant change in use of care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to Report

6. **PRODUCTS:**

Publications, conference papers, and presentations Cook, T., Raban, R., Umotoy, J., Guatam, A., Muller, R., Jett, M., Muller-Cohn, J., and Diaz, P.W. "Establishment of an All Ambient Automated Workflow Biobank Pilot Plant for the Purification and Archiving of Nucleic Acids Found in Blood for the U.S. Army" (SLAS 2016 poster)

Website(s) or other Internet site(s) Nothing to Report

Technologies or techniques Nothing to Report

Inventions, patent applications, and/or licenses Nothing to Report

Other Products Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individual have worked on the project?

Name	Project Role	Nearest person month worked	Contribution to project
Paul Diaz	P.I.	3	Leadership
Vasco Liberal	P.I.	1	Leadership
Gerald Dodson	Scientist	1	Technical Lead
Joel Desharnais	Chemist	1	Technical Support
Troy Cook	Scientist	6	Technical Support
Jeff Umotoy	Research Associate	2	Technical Support

Has there been a change in the active other support of the PD/PI(s) or senior key personnel since the last reporting period? Nothing to Report

What other organizations were involved as partners?

Organization Name: Bioreclamation IVT

Location of Organization: 123 Frost St., Westberry, NY 11590

Partner's contribution to the project: Provide collection and distribution of human blood samples.

8. SPECIAL REPORTING REQUIREMENTS: N/A

9. APPENDICES:

I. Cost analysis for automated workflow.

II. Workflow SOPs

- A) RGB Sample Tracking
- B) RGB Sample Handling
- C) Purification of RNA
- D) Purification of DNA
- E) UV Quantification of DNA and RNA Samples
- F) Sample Dry Down and Storage

III. Photos of processes and instruments used in workflow.

IV. Preparation of samples to be transferred to Army for result verification

Appendix I. Cost analysis for automated workflow

1) Equipment

Costs of the equipment used in the current workflow is shown in Table 4.

Instrument	Cost
Beckman Coulter Biomek FXp	$152,000
ThermoFisher KingFisher Flex	$45,472
Eppendorf 5804R centrifuge	$10,125
Eppendorf 5804R 48 place rotor	$1,540
Genevac HT-12	$40,300
Genevac HT-12 evaporation racks	$12,720
Biomatrica VLC	$24,000
ThermoScientific 384 well Sample Seal	$72,900
Sample scan barcode reader	$4,017
total	**$363,074**

Table 4. Estimation of equipment costs of the workflow in its current parameters.

2) Consumables

Consumable costs of the current workflow for a single RGB tube are shown in Table 5.

Sample Collection	vendor	catalog #	amount	cost	normalized cost	amount used per sample	cost per sample
RNAgard Blood Tubes	Biomatrica	62201-131	50	$435	$8.70	1 tube	$8.70
						Total	**$8.70**
Manual sample processing							
RNA precipitation buffer	Biomatrica	RP1-001-FG	50	$50.00	$1.00	3mL	$3.00
E&K barcoded 15mL tubes	TubeWriter	TW-SRST57527-1D	6000	$3,495.00	$0.58	2 tubes	$1.17
Caps	TubeWriter	TW-SRST65793	0	$0.00	included w tubes	2 caps	0
						Total	**$21.57**
RNA processing							
Agencourt RNAdvance	Beckman coulter	A35604	384	$1,997.00	$5.20	1 reaction	$5.20
P250 AP96 tips sterile barrier	Beckman coulter	717253	960	$189.04	$0.20	1 tip	$0.20
96 well plates for reagents	Fisher	AB2800	25	$131.00	$5.24	2 plates/2 wells	$0.11
RNA sample plates, Seahorse 96 well	E&K scientific	EK-2076	25	$180.00	$7.20	1 plate/1 well	$0.08
Biotix Elution plates	vwr	89511-216	100	$243.77	$2.44	1 plate/1 well	$0.03
						Total	**$5.60**
DNA processing							
Macherey-Nagel NucleoMag 3mL blood	Macherey-Nagel	744502.1	192	$1,155	$6.02	1/2 reaction	$6.02
KingFisher deep well plate 96 well	Molecular BioProd	95040450	50	$267.30	$5.35	11 wells	$0.61
Biotix elution plates	vwr	89511-216	100	$243.77	$2.44	1 plate/1 well	$0.03
Kingfisher 96 well comb	Molecular BioProd	97002534	100	$528.00	$5.28	1 tip per comb	$0.06
						Total	**$6.71**
Drying process							
384 2d barcoded storage plate	Thermo Scientific	3815	20	$1,998	$99.88	8-384 wells	$2.08
Greiner UV quantitation plate	VWR	82051-348	40	$928.31	$23.21	1 plate/2 wells	$0.48
Beckman Coulter 20 uL tips unfiltered	Beckman Coulter	717255	960	$95.63	$0.10	1 tip	$0.10
RNAstable	Biomatrica	52201-013	10mL	$995	$0.10	4x10uL	$3.98
DNAstable Plus	Biomatrica	52091-026	10mL	$1,105	$0.11	4x12.5 uL	$5.53
						Total	**$12.17**
Total consumable costs per sample							**$37.35**

Table 5. Estimation of consumable costs of the entire current workflow for one RGB tube.

3) Labor

The entire workflow with required ***total man hour time*** for each step is shown in Table 6. This is the man hour time for processing for 96 isolations of DNA and RNA from RGB tubes when 2 users are working in the process simultaneously. The total man hours required for both users is approximately 4.5 hours.

	Man hours for User 1	Man hours for User 2
Manual sample processing	1	1
RNA processing	1	
DNA processing		1
Drying and storage	0.5	
Total hours	**2.5**	**2**

Table 6. Man hour time estimated to complete current workflow for harvesting purified DNA and RNA from 96 samples.

4) Sample Storage

The purified, stabilized samples isolated from this workflow are stored at ambient temperature in Biomatrica VLC units rather than freezers. A breakdown of VLC storage capacity pertaining to this workflow is shown in Table 7, and under this workflow each VLC cabinet has the capacity to house 138,240 donor samples. As the long-term goal set by the Army has been set at 1 million soldier samples, this corresponds to 2 million total samples as there will be an RNA and DNA sample harvested from each soldier. Under the current storage constraints, the entirety of this number of samples could be housed in 15 VLC units (Table 8). Lastly, as shown in Table 9 there is a sizable savings of both money and energy through utilization of dry storage via VLC as compared to traditional cold storage in -80°C freezers. Beyond the electricity used by the freezers themselves, the HVAC requirements of the freezers add an incredible cost both financially as well as with respect to the carbon footprint produced by this storage method. The VLC unit run under standard conditions will pull less than 1% of the electricity used by a -80°C freezer, and this does not include the large HVAC energy requirement of the freezer.

Storage Unit	Capacity		Total soldier storage
1 384-well plate	96 soldier samples	4 replicates	96
1 VLC well	4 384-well plates		384
1 VLC tower	30 VLC wells		11,520
1 VLC unit	12 VLC towers		138,240
Total soldiers/VLC		**RNA or DNA**	**138,240**

Table 7. Army sample capacity breakdown of Biomatrica VLC unit.

Total soldiers	Sample types (RNA/DNA)	Aliquots per sample	Total aliquots in Army goal	Total VLCs needed for Army goal
1,000,000	2	4	8,000,000	15

Table 8. VLC requirement to store DNA and RNA samples for 1 million soldiers.

Component of sample storage process	Traditional -80°C Freezer	Dry Storage
Drying	N/A	0.350 kW/h
Energy Use, Storage	2.45 kW/h	0.010 kW/h
Energy Use, HVAC	45.99 kW/h	0.00 kW/h
Heat Rejection	17,914 BTU	0 BTU
Annual CO2 Use Cost	$2,074	$0
Annual Electrical Use Cost	**$25,978**	**~$0**
Risk of sample loss in the event of power failure	**High**	**Very Low**

Table 9. Estimated energy and monetary savings of dry storage as compared to a standard -80°C freezer.

Appendix II. Workflow SOPs

A) SOP1 – (75-WI-BB01) RGB Sample Tracking

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the tracking of a set of 96 RGB samples during processing for the Army Biobank project.

2.0 Scope. This work instruction is applicable to all personnel executing the RGB sample handling for the Army Biobank project

3.0 Reference Documents.

751-WI-BB03 *Purification and Storage of RNA for the Biobank*

751-WI-BB04 *Purification and Storage of DNA for the Biobank*

4.0 Keywords/Abbreviations.

RGB = RNAgard Blood

5.0 Equipment and Materials.

5.1 Equipment:

Equipment/Apparatus	**Biomatrica ID #**
Biomek FX Control PC	Instrument # 0124
Barcode Reader hand held	
BioMicroLab 2D Barcode Scanner	
48-Tube Racks	

5.2 Materials:

Material/Reagent	**Vendor**	**Product #**
RGB Tubes	Biomatrica	62201-131
15 mL Barcoded Conical Tubes	TubeWriter	TW-SRST57527-1D
15 mL Conical Tube Caps (Stopper)	TubeWriter	TW-SRST65793
SeaHorse 96-Deep Well Plate	e&k scientific	EK-2076
KingFisher 96-Deep Well Plate	Thermo	95040450
Matrix 2D Barcoded 384-Storage Tubes	Thermo	3815

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 Sample Tracking Preparation:

6.1.1 Sort the 96 RGB tubes to be processed into 2 tube racks, 48 tubes in each rack. Label one rack RGB 1 and the other rack RGB 2.

6.1.2 For each RGB tube, prepare 2 x 15 mL barcoded conical tubes. One will be used for RNA precipitation (RNA Tube) and one to collect the supernatant containing DNA (DNA Tube). Arrange these tubes into racks as the RGB tubes were in step 6.1.1. Label the racks for the RNA tubes RNA 1 and RNA 2. Label the racks for the DNA tubes DNA 1 and DNA 2.

6.2 Scan Barcodes into the Sample Tracking Table:

6.2.1 Open the "RGB Sample Tracking Table Template.xlsx" spreadsheet file, located on the Biomek FX control PC and Save As with an appropriate name for the samples (i.e. Biobank Samples mm/dd/yy).

6.2.2 After saving the sample tracking table with a new name, click on the cell under barcode for the RGB Tubes next to tube #1. Scan the barcodes of the RGB tubes in rack RGB 1 starting with the tube at the top of the left most column (corresponds to well A1 of the sample plate) and proceed down each column from left to right. The cursor should advance to the next cell below in the spreadsheet after the barcode is scanned. Continue scanning rack RGB 2 when rack RGB 1 is completed. When rack 2 is completed check that the table shows 96 barcodes for RGB Tubes.

6.2.3 Click on the cell under RNA Tube in the RGB Sample Tracking Table spreadsheet. Scan the barcodes of the RNA Tubes using the same order as the RGB tube barcodes were scanned, beginning with rack RNA 1 and continuing with rack RNA 2. Other cells that need these barcodes will be automatically populated. When rack 2 is completed check that the table shows 96 barcodes for RNA Tubes, RNA Sample Plate Locations, and RNA Sample Storage Locations.

6.2.4 Click on the cell under DNA Tube barcode in the Sample Tracking Table spreadsheet. Scan the barcodes of the DNA Tubes using the same order as the RGB tube barcodes were scanned, beginning with rack DNA 1 and continuing with rack DNA 2. Other cells that need these barcodes will be automatically populated. When rack 2 is completed check that the table shows 96 barcodes for DNA Tubes under Sample Processing Tubes and DNA Sample Storage Locations.

6.2.5 Click on the cell below RNA Sample Plate and above Well: under RNA sample plate locations in the Sample Tracking Table spreadsheet. Scan the barcode for the plate that the re-suspended RNA pellet samples will be transferred to.

6.2.6 Click on the cell below RNA Elution Sample Plate and above Well: under RNA Sample Storage Locations in the Sample Tracking Table spreadsheet. Scan the

barcode for the plate that the eluted RNA samples will be transferred to on the Biomek FX.

6.2.7 Click on the cell below RNA 384 Well Storage Plate and above Well: under RNA Sample Storage Locations in the Sample Tracking Table spreadsheet. Scan the barcode for the 384 well storage plate that the purified RNA samples will be transferred to. Other cells that need this barcode will be automatically populated.

6.2.8 Click on the cell below DNA Elution Sample Plate and above Well: under DNA Sample Storage Locations in the Sample Tracking Table spreadsheet. Scan the barcode for the plate that the DNA elution samples will be transferred to on the KingFisher Flex.

6.2.9 Click on the cell below DNA 384 Well Storage Plate and above Tube Barcodes: under DNA Sample Storage Locations in the Sample Tracking Table spreadsheet. Scan the barcode for the 384 well storage plate that the purified DNA samples will be transferred to. Other cells that need this barcode will be automatically populated.

6.3 Scan 384 Well Storage Plate 2D Barcodes:

6.3.1 Open the Sample Scan Mini software on the Biomek FX control PC. Place the RNA 384 Well Storage Plate on the BioMicroLab 2D Barcode Scanner with well A1 positioned as indicated on the scanner. Click on the Scan & Decode button. When the scan is complete an image of the 2D barcodes for the 384 tubes is displayed. Green boxes around the tubes indicates a good scan, a red box indicates an error. If there is an error check that the bottom of the 384 storage plate and the scanning platform of the scanner are clean before re-scanning.

6.3.2 When a good scan of the RNA 384 Well Storage Plate 2D barcodes is obtained set the folder to save the file in by opening the Output Options window in the Setting dropdown menu. Click on the Output Folder button and select the RNA folder for the destination in the Sample Tracking folder on the C drive and click OK to close the window. Click on the Output button below the plate image and give the file an appropriate name for the samples (i.e. mm/dd/yy RNA Sample Storage 2D barcodes) and click the Create button to save.

6.3.3 Open the newly saved 2D barcode file in Excel. Select the 2D barcodes in column C and copy. Paste the barcodes in the "RNA 384 Well Storage Plate Tube Barcodes:" column in the Sample Tracking Table spreadsheet.

6.3.4 Place the DNA 384 Well Storage Plate on the BioMicroLab 2D Barcode Scanner with well A1 positioned as indicated on the scanner. Click on the Scan & Decode button. When the scan is complete an image of the 2D barcodes for the 384 tubes is displayed. Green boxes around the tubes indicates a good scan, a red box indicates an error. If there is an error check that the bottom of the 384 storage plate and the scanning platform of the scanner are clean before re-scanning.

6.3.5 When a good scan of the DNA 384 Well Storage Plate 2D barcodes is obtained set the folder to save the file in by opening the Output Options window in the Setting dropdown menu. Click on the Output Folder button and select the DNA folder for the destination in the Sample Tracking folder on the C drive and click OK to close the

window. Click on the Output button below the plate image and give the file an appropriate name for the samples (i.e. mm/dd/yy DNA Sample Storage 2D barcodes) and click the Create button to save.

6.3.6 Open the newly saved 2D barcode file in Excel. Select the 2D barcodes in column C and copy. Paste the barcodes in the "DNA 384 Well Storage Plate Tube Barcodes:" column in the Sample Tracking Table spreadsheet.

6.3.7 Save the Sample Tracking Table spreadsheet.

6.3.8 Print sections of the Sample Tracking Table spreadsheet to aid in the manual transfer of samples from tube to tube or from tube to plate.

6.3.9 Proceed with the RGB Sample Handling for the Biobank Project work instruction.

B) SOP2 – (75-WI-BB02) RGB Sample Handling

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the sample handling of the RGB tubes for the Biobank project

2.0 Scope. This work instruction is applicable to all personnel executing the RGB sample handling for the Biobank project

3.0 Reference Documents.

751-WI-BB01 *RGB Sample Tracking for the Biobank Project*

751-WI-BB03 *Purification and Storage of RNA for the Biobank*

751-WI-BB04 *Purification and Storage of DNA for the Biobank*

4.0 Keywords/Abbreviations.

RGB = RNAgard Blood

5.0 Equipment and Materials.

5.1 Equipment:

Equipment/Apparatus	**Biomatrica ID #**
Biomek FX	Instrument # 0124
New Brunswick innova 4330 Incubator	Instrument # 0096
Thermo Scientific SampleSeal	Instrument # 0122
Eppendorf 5804 R Centrifuge	Instrument # 0150
Rotor for Eppendorf Centrifuge F-35-48-17	
Tube Rack	
VWR Mini Shaker	Instrument # 0063

5.2 Materials:

Material/Reagent	Vendor	Product #
RGB Tubes	Biomatrica	62201-131
15 mL Barcoded Conical Tubes	TubeWriter	TW-SRST57527-1D
15 mL Conical Tube Caps(Stopper)	TubeWriter	TW-SRST65793
Precipitation Buffer	Biomatrica	RP1-001-FG
Resuspension Buffer	Biomatrica	Custom item
Seahorse 96-Deep Well Plate	e&k scientific	EK-2076

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 Sample Preparation:

6.1.1 If the 96 RGB tubes in racks RGB 1 and RGB 2, from the work instruction for RGB Sample Tracking for the Biobank Project, have been stored at 4°C, let the tubes warm up to ambient temperature for at least 30 minutes before processing.

6.1.2 Add 3 mL of Precipitation Buffer to the 96 RNA Tubes in racks RNA 1 and RNA 2 from the work instruction for RGB Sample Tracking for the Biobank Project (can done ahead of time).

6.2 Blood Tube Processing for 96 RGB Tubes:

6.2.1 The RGB tubes in rack RGB 1 will go through steps 6.2.2 to 6.2.5 first and the RGB tubes in RGB 2 can be processed using the same steps during the centrifugation of the tubes in rack RGB 1.

6.2.2 After inverting the RGB tubes 5 times, transfer the contents of the RGB tubes into the corresponding RNA Tubes and place caps on the RNA tubes. Use a print out of the Sample Tracking Table spreadsheet, from the work instruction for RGB Sample Tracking for the Biobank Project, to aid in the manual transfer of samples from tube to tube. Invert the RNA Tubes 5 times.

6.2.3 Dispose of the empty RGB tubes and caps in an appropriate waste container.

6.2.4 Place rack RNA 1 horizontally into the container in the New Brunswick incubator with the thin Styrofoam sheet between the tube caps and the side of the container. Place the empty section of the rack against one side of the container and place a Styrofoam block between the other side of the container and the rack. Shake at 500 RPM for 6 minutes at ambient temperature.

6.2.5 Within 10 minutes after shaking has been completed, place the RNA Tubes from rack RNA 1 in sequence into the centrifuge (up to 48 tubes will fit in the rotor) and centrifuge at ambient temperature for 40 minutes at 5500 RPM (5005 rcf). After centrifugation place the tubes back into rack RNA 1 in their original sequence. Repeat step with rack RNA 2.

6.2.6 The RNA tubes in rack RNA 1 will go through steps 6.2.7 to 6.3.5 while the RNA tubes from rack RNA 2 are in the centrifuge. The RNA tubes in rack RNA 2 will go through steps 6.2.7 to 6.3.5 after centrifugation.

6.2.7 De-cap all the tubes in rack DNA 1 and place them in a clean container for recapping. Repeat with rack DNA 2 when rack RNA 2 tubes are done with centrifugation.

6.2.8 Open the cap of the RNA Tube, from rack RNA 1, and transfer slowly the supernatant into the corresponding DNA Tube from rack DNA 1. Place the DNA tube back in its location in rack DNA 1 and cap it. Use a printout of the Sample Tracking Table spreadsheet, from the work instruction for RGB Sample Tracking for the Biobank Project, to aid in the manual transfer of samples from tube to tube. Repeat with racks RNA 2 and DNA 2 when rack RNA 2 tubes are done with centrifugation.

6.2.9 Recap the RNA Tube now containing only the RNA pellet and set upside down in its original location in rack RNA 1 so that the remaining liquid will drain into the cap. Repeat steps 6.2.8 and 6.2.9 until the supernatant of all RNA tubes in the rack is transferred to all corresponding DNA tubes. Repeat with rack RNA 2 and DNA 2 when rack RNA 2 tubes are done with centrifugation.

6.2.10 Check the RNA pellets in the inverted tubes periodically for sliding as step 6.2.9 is performed. If a pellet starts to slide take the tube before the pellet reaches the cap and perform step 6.3.1. If the pellet reaches the cap, turn the tube right side up so that the pellet returns to the bottom of the tube and replace the cap with a new one.

6.2.11 The DNA tubes in racks DNA 1 and DNA 2 will be processed using work instruction 751-WI-BB04.

6.3 <u>RNA Resuspension and Transfer</u>:

6.3.1 Take the inverted RNA Tubes in rack RNA 1 and while keeping inverted, uncap and place the caps in a waste container. Place the RNA Tubes back in the rack right side up in their correct locations.

6.3.2 Add 350 µL of Resuspension Buffer to the RNA Tubes in rack RNA 1 and cap the RNA Tubes with new caps. As the tubes are capped tilt each tube so that any part of the RNA pellet that may be up the side of the tube is wetted by the Resuspension Buffer.

6.3.3 Shake the RNA Tubes in rack RNA 1 at 1000 RPM vertically straight up for 10 minutes on the Mini Shaker at ambient temperature. Use clips to hold the rack firmly to the shaker platform while making sure that the tubes are held solidly in the rack.

6.3.4 Transfer the sample from the RNA tubes to the corresponding wells in the RNA Plate. The RNA samples will be transferred to the RNA Plate by column starting with well A1 then B1 and C1 and so forth until all RNA samples are transferred from the RNA Tubes to the RNA Plate. Use a print out of the Sample Tracking Table spreadsheet, from the work instruction for RGB Sample Tracking for the Biobank Project, to aid in the manual transfer of samples from tube to plate.

6.3.5 Keep the RNA Plate on ice while transferring samples from rack RNA 1. Repeat steps 6.2.7 – 6.3.5 for samples from rack RNA 2 when they are done with centrifugation. Continue transferring the RNA samples from the last filled well.

6.3.6 The RNA samples in the RNA Plate are now ready for processing on the Biomek FX using work instruction 751-WI-BB03.

C) SOP3 – (75-WI-BB03) Purification of RNA

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the purification and storage of RNA from 96 RGB tubes using the Biomek FX for the Biobank project

2.0 Scope. This work instruction is applicable to all personnel purifying and storing of RNA for the Biobank project

3.0 Reference Documents.

751-WI-BB02 *RGB Sample handling for the Biobank Project*

751-WI-BB05 *UV Quantification of DNA and RNA Samples for the Biobank Project*

751-WI-BB06 *Sample dry down and storage for the Biobank Project*

4.0 Keywords/Abbreviations.

RGB = RNAgard Blood

5.0 Equipment and Materials.

5.1 Equipment:

Equipment/Apparatus	Biomatrica ID #
Biomek FX	Instrument # 0124
Biomek 3000	Instrument # 0075
Alpaqua Magnum EX Magnet	
BioTek Plate Reader	Instrument # 0281
GeneVac HT12	Instrument # 0123
VLC	

5.2 Materials:

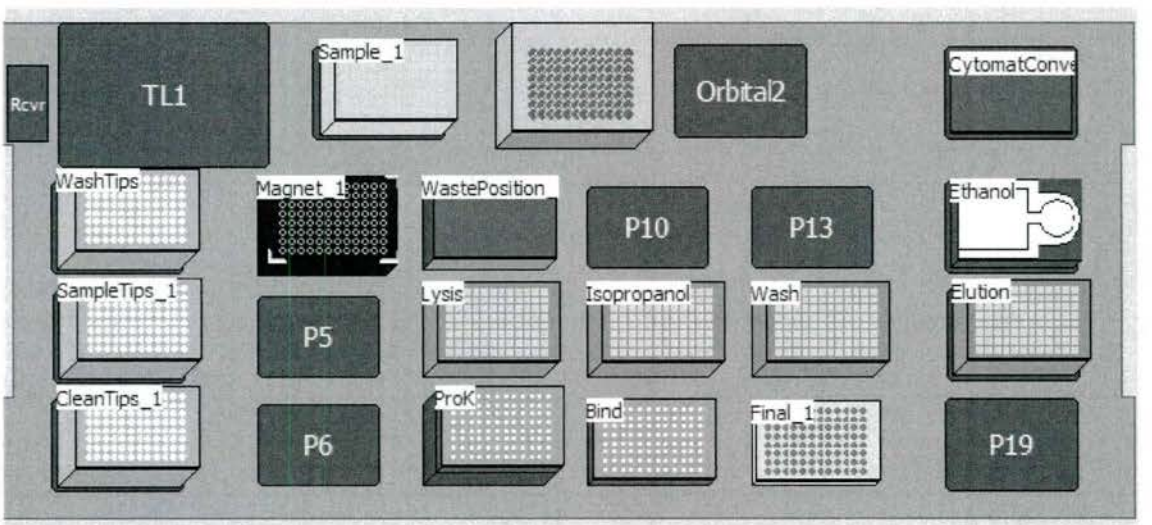
Material/Reagent	Vendor	Product #
P250 Tips	Beckman Coulter	717253
RNAdvance Agencourt Lysis Buffer	Beckman Coulter	A35604
RNAdvance Agencourt Wash Buffer	Beckman Coulter	A35604
RNAdvance Agencourt Proteinase K	Beckman Coulter	A35604
RNAdvance Agencourt Proteinase K Buffer	Beckman Coulter	A35604
RNAdvance Agencourt Binding Beads/Buffer (Bind1)	Beckman Coulter	A35604
Elution Buffer (Nuclease-free water)	USB	71786
Ethanol 200 Proof	Acros	61509-0020
Isopropanol	EMD	PX1835-5
Riplate 2.2 mL Deepwell 96 plate	Ritter	43001-0020
Biotix 96-Well 350 µL u-bottom plate	VWR	89511-216
Matrix Storage Tubes 384-Well Plate	Thermo Scientific	3815
ABgene 96-Well PCR Plate	Fisher Scientific	AB2800
Greiner 384 Well Microplate, UV-Star	VWR	82051-348
Seahorse 96-DeepWell Plate, Square,	E&K Scientific	EK-2076

Pyramid Bottom, 2 mL		
SeaHorse Pyramid bottom reservoir	E&K Scientific	EK-2035
Biomek Seal & Sample Foil Seal	Beckman Coulter	538619
RNAstable LD	Biomatrica	52201-013
RNAse Away	Sigma-Aldrich	83931

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 Schematic of Biomek FX Plate Holders:



6.1.1 Before starting to use the Biomek FX, wipe all surfaces of the instrument clean with RNAse away solution.

6.2 Preparation of Reagents:

The reagent reservoirs for the Biomek FX RNA purification method can be filled and placed at their positions on the Biomek FX work surface during the centrifugation of the RNA tubes from rack RNA 1 in Work instruction BB02 step 6.2.5.

6.2.1 Fill a SeaHorse pyramid bottom reservoir with 31 mL of Lysis Buffer and place at position P8.

6.2.2 Fill a SeaHorse pyramid bottom reservoir with 50 mL of Isopropanol and place at position P11.

6.2.3 Fill a SeaHorse pyramid bottom reservoir with 79 mL of Wash Buffer and place at position P14.

6.2.4 Fill a SeaHorse pyramid bottom reservoir with 35 mL of Nuclease free water to be used as Elution Buffer and place at position P18.

6.3 RNA Purification on the Biomek FX:

6.3.1 Open the Biomek Software by double clicking the icon on the desktop. If the program is open then go to the next step.

6.3.2 Select ***Open Project*** in the Project drop down menu and Select ***RNAdvance Blood*** in the Select a project to open: portion of the Open Project dialog.

6.3.3 Select ***Open...*** from the File drop down menu and Select ***RNAdvance Blood dual-Plate MC v1.0.1 new plates*** from the Select a method: portion of the Open Method dialog.

6.3.4 Click the Play button to Run the method and the RNAdvance method start window opens.

6.3.5 Set the "Number of Plates" to 1 in the "Plates" section of the "Quick Start" tab in the RNAdvance method start window.

6.3.6 Refer to the "Deck Display" section of the RNAdvance method start window for placement of labware and reagents on the deck of the FX. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.3.7 Put racks of P250 barrier tips into each of the specified deck positions:
- WashTips P1
- SampleTips_1 P2
- CleanTips_1 P3

6.3.8 Make sure the magnets are present at position P4 (Magnet_1).

6.3.9 Place Ritter 96-deep well plates at positions P7 (Biowaste) and P9.

6.3.10 Place an Abgene AB2800 96-well PCR plate containing 25 µL of Proteinase K per well (from a stock solution freshly prepared by mixing 500 mg of Proteinase K stored at -20°C with 10 mL of Proteinase K Buffer) at position P9 on top of the Ritter 96-deep well plate.

6.3.11 Place an Abgene AB2800 96-well PCR plate containing 12 µL of Binding Beads per well onto position P12.

6.3.12 Fill the bottle that feeds the reservoir indicated as "Ethanol" at position P17 with 85% Ethanol (prepared by adding water to ethanol 200 proof) and place the bottle into the reservoir receptacle. Open the valve to fill the reservoir.

6.3.13 Place the SeaHorse Pyramid bottom 96-deep well plate (Sample_1), onto the Orbital1 position of Biomek FX deck.

6.3.14 Place the empty Biotix 96-well polypropylene u-bottom plate (Final_1) onto position P15.

6.3.15 Click the green "Play" arrow at the bottom right of the RNAdvance method start window to start the RNA purification method. The RNAdvance method start window closes and the FX Deck Setup window opens. Click the OK button to close it and proceed with the method. The method will complete in 2 hrs and 15 min.

6.4 RNAstable LD Plating into 384-Well Storage Plate on the Biomek 3000:

While the RNA purification runs on the Biomek FX, the RNAstable LD solution can be dispensed into the 384-well storage plate using the Biomek 3000. Any peristaltic type plate dispenser that can dispense to a 384 well plate may be used.

6.4.1 Open the Biomek Software on the Biomek 3000 by double clicking the icon on the desktop. If the program is open then go to the next step.

6.4.2 Select ***Open Project*** in the Project drop down menu and Select ***Biobank*** in the Select a project to open: portion of the Open Project dialog.

6.4.3 Select ***Open...*** from the File drop down menu and Select ***Transfer RNAstable to Storage Plate*** from the Select a method: portion of the Open Method dialog.

6.4.4 Place the 384-well storage plate onto the P6 position of Biomek 3000 deck. Place the RNAstable reservoir onto the P7 position of Biomek 3000 deck with the shallow reservoir module in the 2nd position from the left of the reservoir rack.

6.4.5 Place P250 tips onto the ML1 position of Biomek 3000 deck.

6.4.6 Click on the Instrument Setup step for the method and the deck configuration appears. Double click on the Tip Box and a Labware Properties window for the tips opens. Click on Show Available Tips and indicate the tips present in the tip box.

6.4.7 Click the "Play" button to Run the method and the Deck Setup window opens. Check that the actual placement of labware and reagents on the deck matches the Deck displayed in the window. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.4.8 Click the "OK" button and the window will close and the method starts. When the method is finished, cover the filled 384-well storage plate with a lid. Rinse the Shallow Reservoir thoroughly and dry it for further use.

6.4.9 Close the method, do not save changes.

6.5 UV Quantification of RNA Samples

6.5.1 Clear the Biomek FX deck of labware from previous method except for the RNA elution plate containing the purified RNA samples.

6.5.2 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer 96 Round Bottom plate to 384 UV*** from the Select a method: portion of the Open Method dialog.

6.5.3 Place the 384-well UV plate onto position P5 of Biomek FX deck. Place the RNA elution plate onto the magnets at position P4 of Biomek FX deck.

6.5.4 Place P20 tips onto the tip loader position of Biomek FX deck.

6.5.5 Right click on the method step for the quadrant of the 384 UV plate that you want to transfer the samples to and select Enable Step from the menu.

6.5.6 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.5.7 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.5.8 After the samples have been transferred to the UV plate, close the method, do not save changes, and take the UV plate to the BioTek Plate reader sample quantification using work instruction 751-WI-BB05.

6.6 Transfer the RNA Samples from the Elution plate to the RNA Storage Plate for Dry Down:

As the RNA quantification runs on the plate reader, the RNA samples in the Elution plate can be transferred to the 384-well RNA Storage plate

6.6.1 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer 96 Round Bottom plate to 384 Storage*** from the Select a method: portion of the Open Method dialog.

6.6.2 Place the 384-well RNA Storage plate with RNAstable LD onto position P5 of Biomek FX deck. Place the RNA elution plate onto the magnets at position P4 of the Biomek FX deck.

6.6.3 Use the same P20 tips from section 6.5 on the tip loader position of Biomek FX deck.

6.6.4 Right click on the method steps for the quadrants of the 384-well RNA Storage plate that were not selected for the quantification transfer and select Enable Steps from the menu.

6.6.5 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.6.6 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.6.7 After the samples have been transferred to the RNA Storage plate, samples from the UV plate will be transferred to the RNA Storage plate.

6.7 Transfer the RNA Samples from the UV plate to the RNA Storage Plate for Dry Down:

After the RNA quantification has finished, the RNA samples need to be transferred from the UV plate to the 384-well RNA Storage plate

6.7.1 Remove the RNA Elution plate the FX deck from previous method

6.7.2 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer UV 384 to 384 Storage*** from the Select a method: portion of the Open Method dialog.

6.7.3 Place the 384-well RNA Storage plate with RNAstable onto position P5 of Biomek FX deck. Place the 384-well UV plate onto the magnets at position P4 of the Biomek FX deck.

6.7.4 Use the same P20 tips from section 6.6 on the tip loader position of Biomek FX deck.

6.7.5 Right click on the method step for the quadrant of the 384-well UV plate that was selected for the quantification transfer and select Enable Step from the menu.

6.7.6 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.7.7 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.7.8 After the samples have been transferred to the RNA Storage plate, remove the RNA Storage plate from the Biomek FX deck and continue processing the plate using sample dry down and storage work instruction 751-WI-BB06.

7.0 Appendix.

7.1 Steps in the Biomek FX RNAdvance RNA Purification Method:

Once the 96-deep well plate is added to position "Sample_1", the Biomek FX will go through the steps presented below:

Default Protocol Settings

Sample	Volume (uL)	400												
ProK	Volume (uL)	20												
Lysis	Volume (uL)	300	Short Shake Seconds	5	Long Shake Minutes	15	Shake RPM	1200						
Isopropanol	Volume (uL)	500												
Bind	Volume (uL)	10	Tipmix Cycles	6	Shake Minutes	2	Shake RPM	900	Incubation Minutes	5	Pulse Shake Seconds	2	Separation Minutes	15
Wash	Volume (uL)	400	Cycles	2	Shake Minutes	3	Shake1 RPM	1300	Shake2 RPM	1000	Separation Minutes	12		
Ethanol	Volume (uL)	750	Cycles	3	Shake Minutes	3	Shake RPM	1100	Separation Minutes	6	Drying Minutes	10		
Elution	Volume (uL)	110	Shake Minutes	3	Shake RPM	1100	Incubation Minutes	1	Separation Minutes	3				
Transfer	Volume (uL)	105												

D) SOP4 – (75-WI-BB04) Purification of DNA

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the utilization of the purification and storage of DNA from RGB tube using the KingFisher for the Biobank project

2.0 Scope. This work instruction is applicable to all personnel purifying and storing of DNA for the Biobank project

3.0 Reference Documents.

751-WI-BB02 *RGB Sample Handling for the Biobank Project*

751-WI-BB05 *UV Quantification of DNA and RNA Samples for the Biobank Project*

751-WI-BB06 *Sample Dry Down and Storage for the Biobank Project*

4.0 Keywords/Abbreviations. N/A

5.0 Equipment and Materials

5.1 Equipment:

Equipment/Apparatus	**Biomatrica ID #**
KingFisher Flex	Instrument # 0273
Biomek FX	
Alpaqua Magnum EX Magnet	
Biomek 3000	Instrument # 0075

5.2 Materials:

Material/Reagent	Vendor	Product #
Binding Buffer	Macherey-Nagel	MBL2
Wash Buffer	Macherey-Nagel	MBL3
Elution Buffer	Macherey-Nagel	MBL5
NucleoMag B-beads	Macherey-Nagel	
96-Deep Well Plate	Thermo	
Standard 96 Well Plate	Thermo	
24 Well Plate	Thermo	
Ethanol		
Seahorse Deep Well Reservoir	e&k scientific	EK-2035
Greiner 384 Well Microplate, UV-Star	VWR	82051-348

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 Fill the KingFisher Processing Plates with Macherey-Nagel Reagents on the Biomek FX:

The Biomek FX method ***Fill All KingFisher RGB process plates with Reagents*** will fill the following plates with reagents:

BindWash plate with 1 mL 75% MBL2 bind buffer per well

SMPL_1 thru SMPL_6 plates with 640 µL MBL2 bind buffer per well

Wash1_1 thru Wash1_3 plates with 1 mL MBL3 wash buffer per well

Wash2_1 plate with 1 mL 80% ethanol per well

Elution plate with a user selected volume of Nuclease-Free Water up to 170 µL.

Place the plates onto the Biomek FX deck with column 1 (the end of the plate with the notched corner) on the left. Method time 14 min. The same can also be accomplished with a Peristaltic type plate dispenser.

6.1.1 Open the Biomek Software by double clicking the icon on the desktop. If the program is open then go to the next step.

6.1.2 Select ***Open Project*** in the Project drop down menu and Select ***RNAdvance Blood*** in the Select a project to open: portion of the Open Project dialog.

6.1.3 Select ***Open...*** from the File drop down menu and Select ***Fill All KingFisher RGB process plates with Reagents*** from the Select a method: portion of the Open Method dialog.

6.1.4 Label a KingFisher 96-Deep Well plate SMPL_1 and place it on the Biomek FX deck at position P19.

6.1.5 Label a KingFisher 96-Deep Well plate SMPL_2 and place it on the Biomek FX deck at position P15.

6.1.6 Label a KingFisher 96-Deep Well plate SMPL_3 and place it on the Biomek FX deck at position P12.

6.1.7 Label a KingFisher 96-Deep Well plate SMPL_4 and place it on the Biomek FX deck at position P9.

6.1.8 Label a KingFisher 96-Deep Well plate SMPL_5 and place it on the Biomek FX deck at position P14.

6.1.9 Label a KingFisher 96-Deep Well plate SMPL_6 and place it on the Biomek FX deck at position P11.

6.1.10 Label a KingFisher 96-Deep Well plate WASH1_1 and place it on the Biomek FX deck at position P16.

6.1.11 Label a KingFisher 96-Deep Well plate WASH1_2 and place it on the Biomek FX deck at position P13.

6.1.12 Label a KingFisher 96-Deep Well plate WASH1_3 and place it on the Biomek FX deck at position P10.

6.1.13 Label a KingFisher 96-Deep Well plate WASH2_1 and place it on the Biomek FX deck at position P6.

6.1.14 Label a KingFisher 96-Deep Well plate BINDWASH and place it on the Biomek FX deck at position P7.

6.1.15 Label a KingFisher 96-Deep Well plate Elution and place it on the Biomek FX deck at position P3.

6.1.16 Label a P200 barrier tips Bind Tips and place it on the Biomek FX deck at position P4.

6.1.17 Label a P200 barrier tips Wash Tips and place it on the Biomek FX deck at position Orbital1.

6.1.18 Label a P200 barrier tips Elution Tips and place it on the Biomek FX deck at position Orbital2.

6.1.19 Label a deep well reservoir Nuclease-Free Water and place it on the Biomek FX deck at position P2 and fill it with 50 mL of Nuclease-Free Water.

6.1.20 Label a deep well reservoir 80% EtOH and place it on the Biomek FX deck at position P5 and fill it with 120 mL of 80% ethanol (prepared by diluting 200 proof ethanol with nuclease-free water).

6.1.21 Label a deep well reservoir labeled 75% MBL2 and place it on the Biomek FX deck at position P8 and fill it with 120 mL of 75% MBL2 (prepared by diluting the binding buffer MBL2 with nuclease-free water).

6.1.22 Label a deep well reservoir Tip Rinse NF Water and place it on the Biomek FX deck at position P20 and fill it with 300 mL of Nuclease-Free Water.

6.1.23 Fill a gravity feed reservoir bottle with 320 mL of MBL3 Wash Buffer and with the valve closed, place it on the gravity feed reservoir on the Biomek FX deck at position P17. Open the valve to fill the reservoir.

6.1.24 Fill a gravity feed reservoir bottle with 400 mL of MBL2 Bind Buffer and with the valve closed, place it on the gravity feed reservoir on the Biomek FX deck at position P18. Open the valve to fill the reservoir

6.1.25 Click the "Play" button to Run the method and a window opens that allows the user to set the volume of the Elution buffer which is at 150 µL default. Enter the desired volume (170 µL max) and click OK.

6.1.26 The Deck Setup window opens. Check that the actual placement of labware and reagents on the deck matches the Deck displayed in the window. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.1.27 Click the "OK" button and the window will close and the method starts.

6.1.28 As the method runs the SMPL_1 plate is the second plate to be filled. When plate SMPL_2 begins to be filled, remove plate SMPL_1 from the FX. If the FX light curtain is tripped, click OK on the message that appears and the method will continue.

6.1.29 Dispense 75 µL of Macherey-Nagel NucleoMag B-beads into each well of plate SMPL_1.

6.1.30 When the method finishes take the SMPL plates to the Biomek 3000 for sample transfers. Take the other plates to the KingFisher.

6.1.31 The reservoirs and bottles and valves should be thoroughly rinsed and dried for further use.

6.2 Transferring the DNA Tube Samples to the KingFisher Sample Plates on the Biomek 3000:

The Biomek 3000 will dispense 320 µL of each DNA Tube to its designated well on each of the 6 KingFisher sample plates pre-loaded with reagents. The DNA Tubes, containing the RGB DNA supernatant, are to be loaded into KingFisher 24 well plates, to be used as tube racks (column 1 is at the end of the plate with the notched corner), as follows:

From rack DNA 1:

Column 1 to 24 well plate 1 RGB 24, well A1, B1, C1, D1, A2, B2, C2, D2

Column 2 to 24 well plate 1 RGB 24, well A3, B3, C3, D3, A4, B4, C4, D4

Column 3 to 24 well plate 1 RGB 24, well A5, B5, C5, D5, A6, B6, C6, D6

Column 4 to 24 well plate 2 RGB 24, well A1, B1, C1, D1, A2, B2, C2, D2

Column 5 to 24 well plate 2 RGB 24, well A3, B3, C3, D3, A4, B4, C4, D4

Column 6 to 24 well plate 2 RGB 24, well A5, B5, C5, D5, A6, B6, C6, D6

From rack DNA 2:

Column 7 to 24 well plate 3 RGB 24, well A1, B1, C1, D1, A2, B2, C2, D2

Column 8 to 24 well plate 3 RGB 24, well A3, B3, C3, D3, A4, B4, C4, D4

Column 9 to 24 well plate 3 RGB 24, well A5, B5, C5, D5, A6, B6, C6, D6

Column 10 to 24 well plate 4 RGB 24, well A1, B1, C1, D1, A2, B2, C2, D2

Column 11 to 24 well plate 4 RGB 24, well A3, B3, C3, D3, A4, B4, C4, D4

Column 12 to 24 well plate 4 RGB 24, well A5, B5, C5, D5, A6, B6, C6, D

6.2.1 Open the Biomek Software on the Biomek 3000 by double clicking the icon on the desktop. If the program is open then go to the next step.

6.2.1 Select ***Open Project*** in the Project drop down menu and Select ***Biobank*** in the Select a project to open: portion of the Open Project dialog.

6.2.2 Select ***Open...*** from the File drop down menu and Select ***Transfer 96 RGB DNA Samples to Sample plates*** from the Select a method: portion of the Open Method dialog.

6.2.3 Place the tool rack at position Rack1 and place the P1000L pipette tool in the rack in the second position from the left.

6.2.4 Place a box of P1000 tips at position PT1.

6.2.5 Place plate SMPL-1 position B1.

6.2.6 Place plate SMPL-2 position B2.

6.2.7 Place plate SMPL-3 position B3.

6.2.8 Place plate SMPL-4 position A3.

6.2.9 Place plate SMPL-5 position A4.

6.2.10 Place plate SMPL-6 position A5.

6.2.11 Place tube rack 1 RGB 24 at position A6.

6.2.12 Place tube rack 2 RGB 24 at position B4.

6.2.13 Place tube rack 3 RGB 24 at position B5.

6.2.14 Place tube rack 4 RGB 24 at position B6.

6.2.15 Click the "Play" button to Run the method and the Deck Setup window opens. Check that the actual placement of labware and reagents on the deck matches the Deck displayed in the window. If needed the PC mouse cursor

can be placed over any deck location to display a description of what should be there.

6.2.16 Click the "OK" button and the window will close and the method starts.

6.2.17 Halfway through the method, about 45 min, the method will pause so that a new box of P1000 tips can be placed on the deck. Click OK when the new box of P1000 tips is placed on the deck and the method will continue.

6.2.18 When the method finishes take the sample plates for DNA purification on the KingFisher. Cap the DNA Tubes and store.

6.3 DNA Purification on the KingFisher:

DNA samples from all 96 DNA Tubes will be purified on the KingFisher Flex with the ***RGB DNA Purification with Macherey-Nagel 3mL Blood Reagents*** protocol using the six 96-Deep Well sample plates and reagent plates that were prepared on the Biomek FX. Each DNA sample was transferred from its tube to its designated well on each of the six sample plates on the Biomek 3000 giving 6 splits for each sample that will be combined during the magnetic bead binding process.

6.3.1 Open the Bandit 3.3.1 software for the KingFisher by double clicking the desktop icon. If the program is open then go to the next step.

6.3.2 Select the ***RGB DNA Purification with Macherey-Nagel 3mL Blood Reagents*** protocol from the ***Home*** tab in the ***Recent Protocols*** list

6.3.3 Click on the ***Start*** button to begin the protocol.

6.3.4 Follow the instructions on the screen of the control panel for loading the plates onto the KingFisher. Load the plates with column 1 (the end of the plate with the notched corner) facing in toward the middle of the turn table.

6.3.5 The protocol will take 8 of the 12 plates to begin with, once the 8 plates are loaded, the automated purification process will begin. After 1 hour 20 min the protocol will signal for the exchange of 4 plates for the remaining 4 sample plates. Total time for the run should be about 2 hours 10 minutes.

6.4 Transfer of DNAstable Plus into 384-Well Storage Plate on the Biomek 3000:

While the DNA purification runs on the KingFisher, the DNAstable Plus for drying the purified DNA can be dispensed into the 384-well storage plate using the Biomek 3000. Any peristaltic type plate dispenser that can dispense to a 384-well plate may also be used.

6.4.1 Open the Biomek Software on the Biomek 3000 by double clicking the icon on the desktop. If the program is open then go to the next step.

6.4.2 Select ***Open Project*** in the Project drop down menu and Select ***Biobank*** in the Select a project to open: portion of the Open Project dialog.

6.4.3 Select ***Open...*** from the File drop down menu and Select ***Transfer DNAstable to Storage Plate*** from the Select a method: portion of the Open Method dialog.

6.4.4 Place the 384-well storage plate onto the P6 position of Biomek 3000 deck. Load a reservoir with 10 mL of DNAstable LD and place onto the P7 position of Biomek 3000 deck with the shallow reservoir module in the 2nd position from the left of the reservoir rack.

6.4.5 Place P250 tips onto the ML1 position of Biomek 3000 deck.

6.4.6 Click on the Instrument Setup step for the method and the deck configuration appears. Double click on the Tip Box and a Labware Properties window for the tips opens. Click on Show Available Tips and indicate the tips present in the tip box.

6.4.7 Click the "Play" button to Run the method and the Deck Setup window opens. Check that the actual placement of labware and reagents on the deck matches the Deck displayed in the window. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.4.8 Click the "OK" button and the window will close and the method starts. When the method is finished, cover the filled 384-well storage plate with a lid and take it to the Biobank. Rinse the Shallow Reservoir thoroughly and dry it for further use.

6.4.9 Close the method, do not save changes.

6.5 Transfer of DNA Samples for UV Quantification

6.5.1 Clear the FX deck of labware from previous methods.

6.5.2 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer 96 Round Bottom plate to 384 UV*** from the Select a method: portion of the Open Method dialog.

6.5.3 Place the 384-well UV plate onto position P5 of Biomek FX deck. Place the DNA elution plate onto the magnets at position P4 of Biomek FX deck.

6.5.4 Place P20 tips onto the tip loader position of Biomek FX deck.

6.5.5 Right click on the method step for the quadrant of the 384-well UV plate that you want to transfer the samples to and select Enable Step from the menu.

6.5.6 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.5.7 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.5.8 After the samples have been transferred to the UV plate, close the method, do not save changes, and take the UV plate to the Biotek Plate reader sample quantification using work instruction 751-WI-BB05.

6.6 Transfer the DNA Samples from the Elution plate to the Storage Plate for Dry Down:

As the DNA quantification runs on the plate reader, the DNA samples in the Elution plate can be transferred to the 384-well storage plate

6.6.1 Clear the FX deck of labware from previous methods.

6.6.2 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer 96 Round Bottom plate to 384 Storage*** from the Select a method: portion of the Open Method dialog.

6.6.3 Place the 384-well storage plate with DNAstable onto position P5 of Biomek FX deck. Place the DNA elution plate onto the magnets at position P4 of the Biomek FX deck.

6.6.4 Use the same P20 tips from section 6.4 on the tip loader position of Biomek FX deck.

6.6.5 Right click on the method steps for the quadrants of the 384 storage plate that were not selected for the quantification transfer and select Enable Steps from the menu.

6.6.6 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.6.7 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.6.8 After the samples have been transferred to the Storage plate, prepare for the transfer of samples from the UV plate to the Storage plate as indicated in section 6.6.

6.7 Transfer the DNA Samples from the UV plate to the Storage Plate for Dry Down:

After the DNA quantification has finished, the DNA samples need to be transferred from the UV plate to the 384-well Storage plate

6.7.1 Clear the FX deck of labware from previous methods.

6.7.2 Select ***Open...*** from the File drop down menu and Select ***Sample Transfer UV 384 to 384 Storage*** from the Select a method: portion of the Open Method dialog.

6.7.3 Place the 384-well Storage plate with DNAstable LD onto position P5 of Biomek FX deck. Place the 384-well UV plate onto the magnets at position P4 of the Biomek FX deck.

6.7.4 Use the same P20 tips from section 6.5 on the tip loader position of Biomek FX deck.

6.7.5 Right click on the method step for the quadrant of the 384-well UV plate that was selected for the quantification transfer and select Enable Step from the menu.

6.7.6 Click the Play button to Run the method and the Deck Setup window opens. Refer to the Deck displayed in the window for placement of labware on the deck. If needed the PC mouse cursor can be placed over any deck location to display a description of what should be there.

6.7.7 Click the OK button in the Deck Setup window and the window will close and the method starts.

6.7.8 After the samples have been transferred to the Storage plate, remove the storage plate from the Biomek FX deck and continue processing the plate using sample dry down and storage work instruction 751-WI-BB06.

7.0 Appendix.

7.1 Description of the automated steps:

When the KingFisher protocol ***RGB DNA Purification with Macherey-Nagel 3mL Blood Reagents*** is started, the DNA samples will go through the following steps:

Reagent info

SMPL_1		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
BINDING BUFFER	640	-	Reagent
Beads ADD 75 ul	40	-	Reagent

SMPL_2		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
Binding Buffer	640	-	Reagent

SMPL_3		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
Binding Buffer	640	-	Reagent

SMPL_4		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
Binding Buffer	640	-	Reagent

Wash 1_1		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
MBL3	1000	-	Reagent

Wash 1_2		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
MBL3	1000	-	Reagent

Wash 2_1		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
80% EtOH	1000	-	Reagent

Elution Plate		96 standard plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Nuclease Free Water add up to 170 ul	150	-	Reagent

BindWash		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
75% MBL2	1000	-	Reagent

Wash 1_3		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
MLB3	1000	-	Reagent

SMPL_5		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
Binding Buffer	640	-	Reagent

SMPL_6		96 DW plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
RGB	320	-	Reagent
Binding Buffer	640	-	Reagent

Steps data

Tip1		96 DW tip comb	
	Pick-Up	SMPL_1	
	Bind1	SMPL_1	
	Beginning of step	Precollect	No
		Release beads	No
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	15
	BindWash 1	BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:00:20, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Bind2	SMPL_2	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	20
	BindWash 2	BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:00:30, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30

	Bind3	SMPL_3	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	25
	BindWash 3	BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:00:40, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Bind4	SMPL_4	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	30
	BindWash 4	BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:00:50, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30

Bind5		SMPL_ 5	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	30
BindWash 5		BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
Bind6		SMPL_ 6	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	30
BindWash 6		BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30

	Wash 1_1	Wash 1_1	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Wash 1_2	Wash 1_2	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Medium
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Wash 1_3	Wash 1_3	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:30, Half mix
		Shake 2 time, speed	00:01:00, Fast
		Shake 3 time, speed	00:00:30, Half mix
		Loop count	3
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Wash 2_1	Wash 2_1	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Shake 1 time, speed	00:00:15, Half mix
		Shake 2 time, speed	00:00:30, Fast
		Shake 3 time, speed	00:00:15, Half mix
		Loop count	2
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	2
		Collect time [s]	30
	Dry1	Wash 2_1	
		Dry time	00:10:00
		Tip position	Outside well / tube

	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Slow
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	72
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	30
	Retire Beads 1	BindWash	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:02:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect beads	No
	Leave	Wash 2_1	

E) SOP5 – (75-WI-BB05) UV Quantification of RNA and DNA Samples

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the UV quantification of DNA and RNA samples for the Biobank project

2.0 Scope. This work instruction is applicable to all personnel executing the UV quantification of DNA and RNA samples for the Biobank project

3.0 Reference Documents.

751-WI-BB03 *Purification of RNA for the Army Biobank project*

751-WI-BB04 *Purification of DNA for the Army Biobank project*

4.0 Keywords/Abbreviations.

RGB = RNAgard Blood

5.0 Equipment and Materials

5.1 Equipment:

Equipment/Apparatus	Biomatrica ID #
Biotek Synergy HTX Plate Reader	Instrument # BM 0280

5.2 Materials:

Material/Reagent	Vendor	Product #
Greiner 384 Well Microplate, UV-Star	VWR	82051-348

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 RNA and DNA Sample UV Quantification:

6.1.1 Pipette 60 µL of Nuclease-Free Water, to be used as blanks, into wells B1, D1, F1, H1, J1, L1, N1, and P1 of the 384-well UV plate that has either purified RNA samples from work instruction 751-WI-BB03 or purified DNA samples from work instruction 751-WI-BB04.

6.1.2 Open the Gen 5 v2.09 control software for the Biotek Synergy HTX plate reader.

6.1.3 Select ***Experiments*** in the Task Manager window and click on ***Open***. Go to the Biobank folder under UV DATA on the C drive. Select one of the ***A260A280 DNA Concentration_.xpt*** experiment files for DNA samples, or one of the ***A260A280 RNA Concentration_.xpt*** experiment files for RNA samples. Select Save As and save the experiment with a new name.

6.1.4 Select Plate Layout under Protocol in the File menu and double click to open the Plate Layout window. Make any adjustments to the plate layout needed to reflect the locations of the blanks and samples on the 384-well UV plate. Click OK to save the layout and close the window.

6.1.5 Select Procedure under Protocol in the File menu and double click to open the Procedure window. Double click on the Read step to open the Read Step window. Click on the button in the upper right of the window to open the Read Range window and select the read range for the 384 UV plate. Click OK to save the Read Range and close the window. Click OK to save the Read step and close the window. Click OK to save the Protocol and close the window.

6.1.6 Click on the Read button to read the plate. Place the plate on the plate carrier when prompted and click on OK to start the read.

6.1.7 When the plate read has completed Click on the Data field at the top of the experiment window and select 260. Click on the Excel button next to the Data field and the raw 260 data is placed in an Excel spreadsheet. Repeat for 280 data, 320 data, and Pathlength data. Save the UV data spreadsheet.

6.1.8 Take the 384-well UV sample plate back to the Biomek FX to complete the sample storage transfers for either purified RNA samples for work instruction 751-WI-BB03 or purified DNA samples for work instruction 751-WI-BB04.

6.1.9 Open the Concentration Template Excel spreadsheet needed for the sample type. The template names are; 384 A260A280 DNA Concentration Blank Correction Template, and 384 A260A280 RNA Concentration Blank Correction Template.

6.1.10 Open the UV data spreadsheet and paste the UV data for the samples into the Concentration Template in Raw Data Tab. The sample concentrations are automatically calculated.

6.1.11 Close the UV data spreadsheet. Click Save As for the template file and select the UV data file as the new name and save the template overwriting UV data file.

F) SOP6 – (75-WI-BB06) Sample Dry Down and Storage

1.0 Purpose. The purpose of this work instruction is to provide step-by-step instructions for the dry down and storage of the purified nucleic acids for the Biobank project

2.0 Scope. This work instruction is applicable to all personnel executing the sample dry down and storage for the Biobank project

3.0 Reference Documents.

751-WI-BB03 *Purification of RNA for the Biobank project*

751-WI-BB04 *Purification of DNA for the Biobank project*

4.0 Keywords/Abbreviations.

RGB = RNAgard Blood

5.0 Equipment and Materials

5.1 Equipment:

Equipment/Apparatus	**Biomatrica ID #**
GeneVac HT12	Instrument # 0123
Thermo Scientific SampleSeal	Instrument # 0122
VLC	

5.2 Materials:

Material/Reagent	**Vendor**	**Product #**
384-Well Storage Plate	Thermo	3815

5.3 Equipment Acceptance Criteria: All equipment used in this work instruction must be maintained and calibrated per the manufacturer's instructions and per Standard Operation Procedure (SOP) 760, *Inspection, Measuring, & Test Equipment Controls.*

6.0 Instructions.

6.1 RNA and DNA Sample Dry Down and Storage:

6.1.1 Put the purified RNA sample storage plate, from Work Instruction 751-WI-BB03 and /or purified DNA sample storage plate, from Work Instruction 751-WI-BB04 into the plate carrier of the GeneVac.

6.1.2 Start the program called "Biobank" on the GeneVac.

6.1.3 Power up the Thermo Scientific SampleSeal plate sealer and press the reset button while the samples are drying in the GeneVac.

6.1.4 Remove the 384-well storage plate from the GeneVac once the program has ended.

6.1.5 Place the 384-well storage plate onto the plate carriage of the SampleSeal plate sealer in the specified orientation and press the "GO" button.

6.1.6 Remove the sealed 384-well storage plate from the SampleSeal plate sealer.

6.1.7 Place the 384-well storage plate into the VLC.

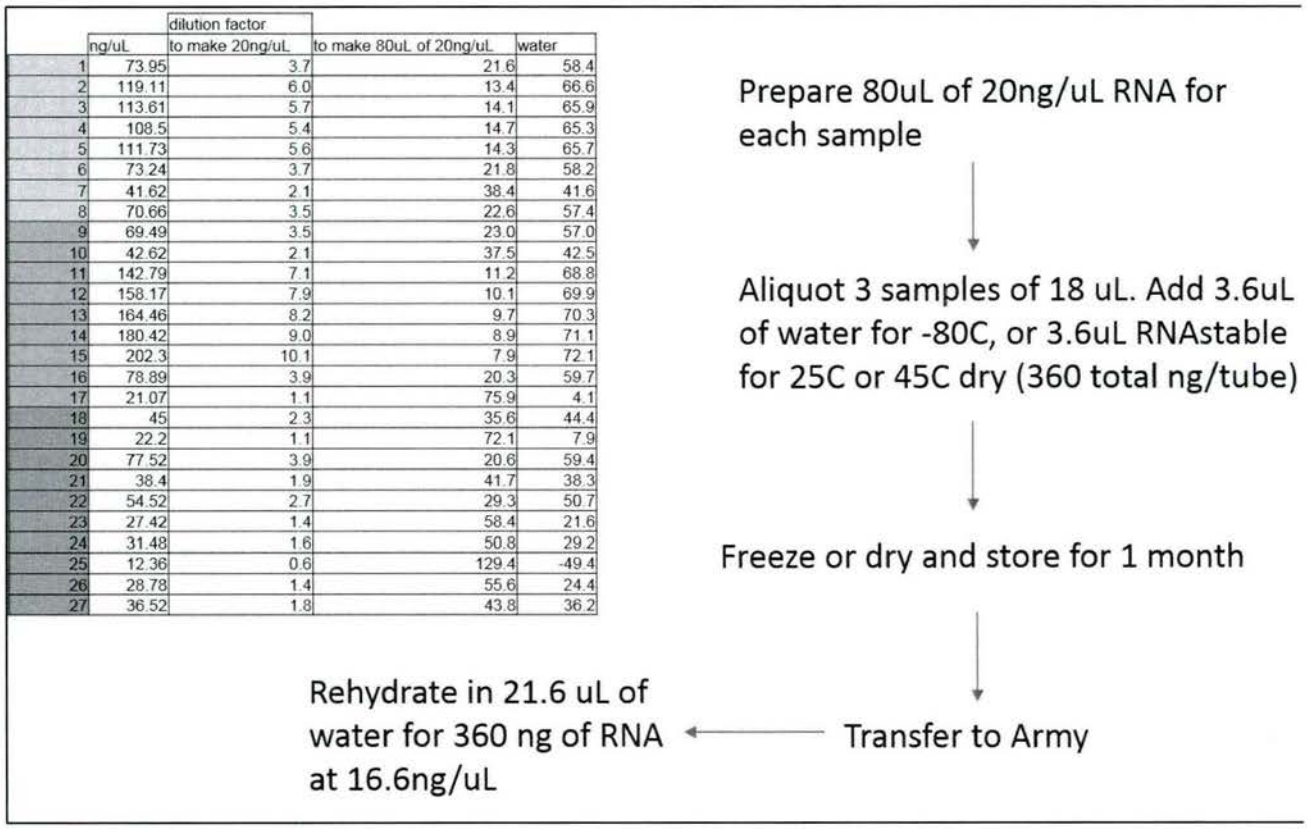
Appendix III. Photos of instruments and processes used in the workflow

Image	Description
A	Hand held scanning of individual sample tubes
B	Configuration of tubes in shaker prior to centrifugation
C	Separation of supernatant from pellet by centrifugation
D	Supernatant and pellet separated into individual sample tubes
E	KingFisher Flex setup for Macherey-Nagel DNA purification
F	Biomek FX setup for Agencourt RNAdvance RNA purification
G	Genevac HT-12 setup for dry down of nucleic acids
H	Storage of dried nucleic acids in VLC after heat sealing

Appendix IV. Preparation of samples to be transferred to Army

Utilizing our method for purification of DNA and RNA from RGB tubes, we have prepared 27 samples from 4 donors (grouped by color) for diagnostic testing by Army. UV quantitation and storage workflows of the purified RNA and DNA are shown in Figure 8 and 9, respectively. Samples were prepared at the desired concentration, dried or frozen, and then the matching samples were stored at 45°C, 25°C, or -80°C for a minimum of 1 month. The map for each matching sample plate (DNA or RNA) is shown in Figure 10.

	ng/uL	dilution factor to make 20ng/uL	to make 80uL of 20ng/uL	water
1	73.95	3.7	21.6	58.4
2	119.11	6.0	13.4	66.6
3	113.61	5.7	14.1	65.9
4	108.5	5.4	14.7	65.3
5	111.73	5.6	14.3	65.7
6	73.24	3.7	21.8	58.2
7	41.62	2.1	38.4	41.6
8	70.66	3.5	22.6	57.4
9	69.49	3.5	23.0	57.0
10	42.62	2.1	37.5	42.5
11	142.79	7.1	11.2	68.8
12	158.17	7.9	10.1	69.9
13	164.46	8.2	9.7	70.3
14	180.42	9.0	8.9	71.1
15	202.3	10.1	7.9	72.1
16	78.89	3.9	20.3	59.7
17	21.07	1.1	75.9	4.1
18	45	2.3	35.6	44.4
19	22.2	1.1	72.1	7.9
20	77.52	3.9	20.6	59.4
21	38.4	1.9	41.7	38.3
22	54.52	2.7	29.3	50.7
23	27.42	1.4	58.4	21.6
24	31.48	1.6	50.8	29.2
25	12.36	0.6	129.4	-49.4
26	28.78	1.4	55.6	24.4
27	36.52	1.8	43.8	36.2



***original concentration of Sample 25 was below 20 ng/uL so 18uL of 12.36 ng/uL was stored. Thus the total RNA stored in each aliquot of this particular sample is 223.2 ng.**

Figure 8. Quantitation and storage workflow of RNA purified from 27 samples.

	ng/uL	Dilution factor to make 31.25 ng/uL	to make 120 uL of 31.25 ng/uL	water
1	68.27	2.18464	54.9	65.1
2	63.69	2.03808	58.9	61.1
3	57.41	1.83712	65.3	54.7
4	71.13	2.27616	52.7	67.3
5	65.8	2.1056	57.0	63.0
6	67.08	2.14656	55.9	64.1
7	51.27	1.64064	73.1	46.9
8	55.32	1.77024	67.8	52.2
9	77.86	2.49152	48.2	71.8
10	81.65	2.6128	45.9	74.1
11	65.45	2.0944	57.3	62.7
12	80.86	2.58752	46.4	73.6
13	74.34	2.37888	50.4	69.6
14	72.96	2.33472	51.4	68.6
15	79.26	2.53632	47.3	72.7
16	76.39	2.44448	49.1	70.9
17	82.26	2.63232	45.6	74.4
18	115.61	3.69952	32.4	87.6
19	115.59	3.69888	32.4	87.6
20	114.04	3.64928	32.9	87.1
21	120.66	3.86112	31.1	88.9
22	114.75	3.672	32.7	87.3
23	72.43	2.31776	51.8	68.2
24	71.21	2.27872	52.7	67.3
25	66.46	2.12672	56.4	63.6
26	72.41	2.31712	51.8	68.2
27	76.05	2.4336	49.3	70.7

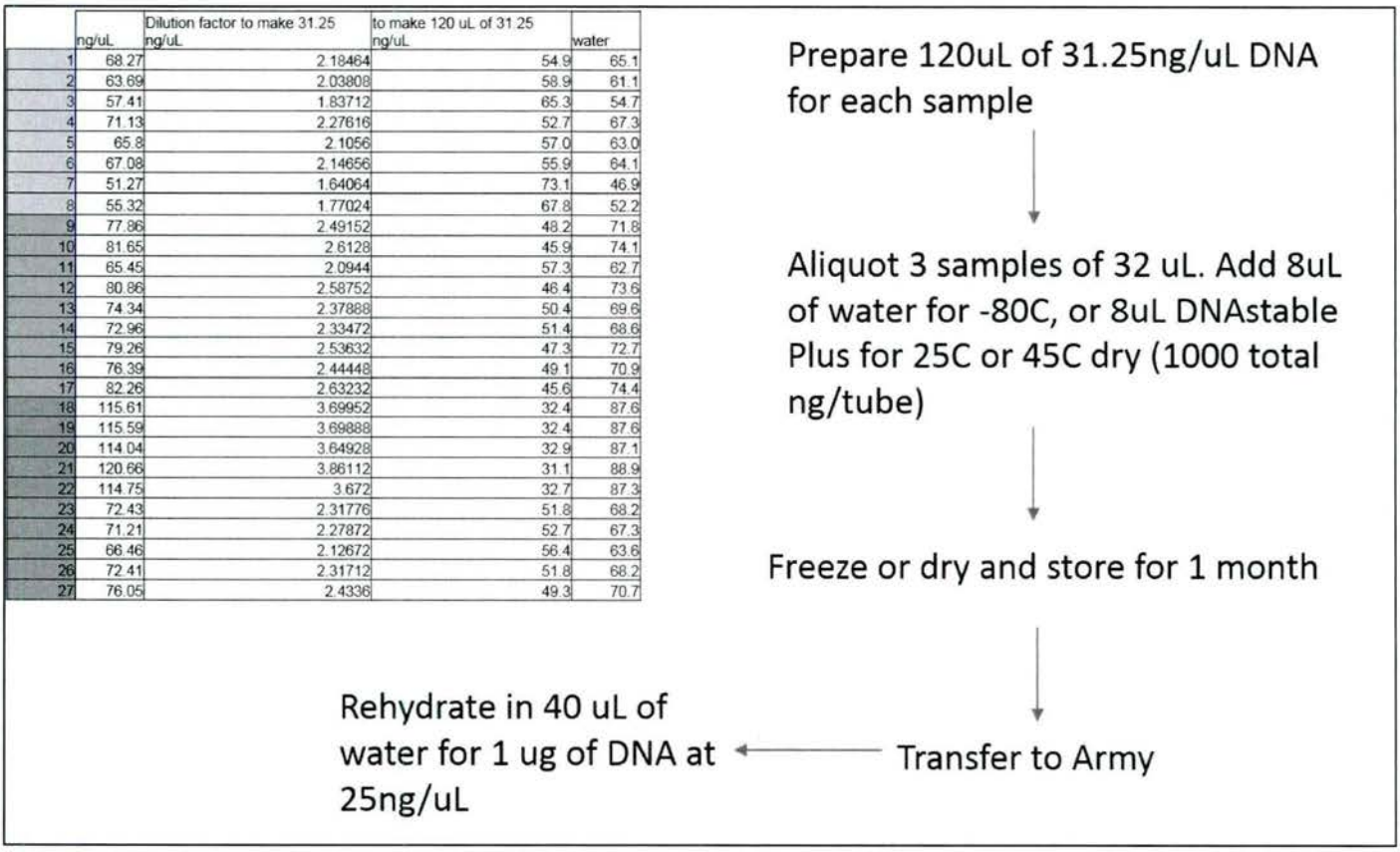


Figure 9. Quantitation and storage workflow of DNA purified from 27 samples

Sample plate layout common to both RNA and DNA samples

Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
B	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24
C	Sample 25	Sample 26	Sample 27	x	x	x	x	x	x	x	x	x
D	x	x	x	x	x	x	x	x	x	x	x	x
E	x	x	x	x	x	x	x	x	x	x	x	x
F	x	x	x	x	x	x	x	x	x	x	x	x
G	x	x	x	x	x	x	x	x	x	x	x	x
H	x	x	x	x	x	x	x	x	x	x	x	x

Figure 10. Map of DNA/RNA sample plates for rehydration and validation testing by Army.